TABLE 2
Insecticidal Activity of Substituted 2-Nitroiminoimidazolidines and Reference Compounds

Pest	*Stage*[b]	*Concentration (mg AI litre*$^{-1}$*)*	*Insecticidal activity*[a]								
			1	***2a***	***2b***	***2c***	***5a***	***5b***	***5c***	***8a***	***8b***
Spodoptera littoralis	L1	100	++	−	−	−	−	−		++	++
Diabrotica balteata	L3	100	++	−	−	−	−	−		++	++
Heliothis virescens	L1	100	++	−	−	−	−	−	−	−	−
Aphis craccivora	m.p.	100	++	−	−	−	−	−	−	−	−
Myzus persicae	m.p.	12·5	++	−	−	−	−	−	−	++	++
Nilaparvata lugens	N3	100	++	−	−	−	−	−	−	++	++
Tetranychus urticae	m.p.	100	−	−	−	−	−	−	−	−	−

[a] ++ >80% mortality; − <30% mortality.
[b] L1: first-instar larvae, L3: third-instar larvae, m.p.: mixed population, N3: third-instar nymph.

D. balteata: Corn seedlings in a Petri dish were treated with test solutions pipetted into the dish and then infested with third-instar larvae. Samples were checked six days after treatment for mortality and growth regulation.

H. virescens: Eggs (0–24 h old) on filter paper were placed in Petri dishes on top of a layer of artificial diet and treated with test solutions introduced by pipette. After six days incubation, samples were checked for egg mortality, larval mortality and growth regulation.

A. craccivora: Pea seedlings, infested with a mixed population were treated with test solutions in a spray chamber. Samples were checked six days after treatment for mortality (contact activity).

M. persicae: Pea seedlings, infested with a mixed population were placed directly in the test solutions. Samples were checked six days after introduction for mortality (systemic activity).

N. lugens: Rice seedlings were treated in a spray chamber with test solution, dried and infested with third-instar nymphs. The samples were checked six to 12 days after the treatment for mortality, growth regulation and effects on F-1 generation.

T. urticae: Bean leaf discs on agar in Petri dishes were infested with a mixed population, and, one day later, were treated with test solution in a spray chamber. Samples were checked for egg mortality, larval mortality, and adult mortality after 10 days incubation.

3 Results and discussion

Table 2 shows the results of insecticidal tests of some new substituted 2-nitroiminoimidazolidines in comparison to imidacloprid (**1**). Compounds **2a**–**c** and **5a**–**c**, were relatively inactive, in contrast to compounds **8a**–**b** which were active at 100 mg AI litre^{-1} (contact) and 12·5 mg AI litre^{-1} (systemic). These results clearly demonstrate that the 5-(2-chloropyridyl)methyl group is necessary for biological activity. The mode of action of **8a**–**b** is probably similar to that of other neonicotinoid insecticides.[6] Further tests were carried out with these compounds, but their activity was clearly poorer than the imidacloprid standard, so the study was discontinued.

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Structure Elucidation of Omphalotin, a Cyclic Dodecapeptide with Potent Nematicidal Activity Isolated from *Omphalotus olearius*

Edwin Büchel,[1] Anke Mayer,[1] Ulrike Martini,[1] Heidrun Anke[1] & Olov Sterner[2]*

[1] LB Biotechnology, University of Kaiserslautern, D-67663 Kaiserslautern, Germany
[2] Department of Organic Chemistry 2, Lund University, PO Box 124, S-22100 Lund, Sweden

Abstract: The structure and absolute configuration of omphalotin, a cyclic dodecapeptide isolated from the basidiomycete fungus *Omphalotus olearius* and possessing potent and selective activity against the plant pathogenic nematode *Meloidogyne incognita*, was determined by a combination of

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Key words: *Omphalotus olearius*; omphalotin A; cyclic dodecapeptide; nematicidal; structure determination; absolute configuration

1 Introduction

Omphalotin exhibits strong nematicidal activity against the plant pathogenic nematode *Meloidogyne incognita* (Kof. & White) Chitwood (LD_{50} 0·57 μM) while being only weakly active against the saprophytic nematode *Caenorhabditis elegans* Maupas (LD_{50} 19 μM).[1] In addition, it is only weakly cytotoxic at 100 μg ml^{-1} and shows no phytotoxic, antibacterial or antifungal activities.[1] Omphalotin, which, following the recent isolation of omphalotins B, C and D, has been renamed omphalotin A,[2] is a cyclic dodecapeptide isolated from cultures of the basidiomycete fungus *Omphalotus olearius* Sing. Initially, the structure was determined by two-dimensional NMR spectroscopy without assignment of the relative or absolute stereochemistry.[3] In this summary, we describe further details about the structure elucidation.

2 Experimental methods

[^{1}H]NMR (500 MHz) and [^{13}C]NMR (125 MHz) spectra were recorded at room temperature with a Bruker ARX500 spectrometer with an inverse multinuclear 5-mm probehead equipped with shielded gradient coil. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine-shaped gradient pulses. For the two-dimensional heteronuclear correlation spectroscopy the refocusing delays were optimised for $^1J_{CH} = 145$ Hz and $^nJ_{CH} = 10$ Hz.

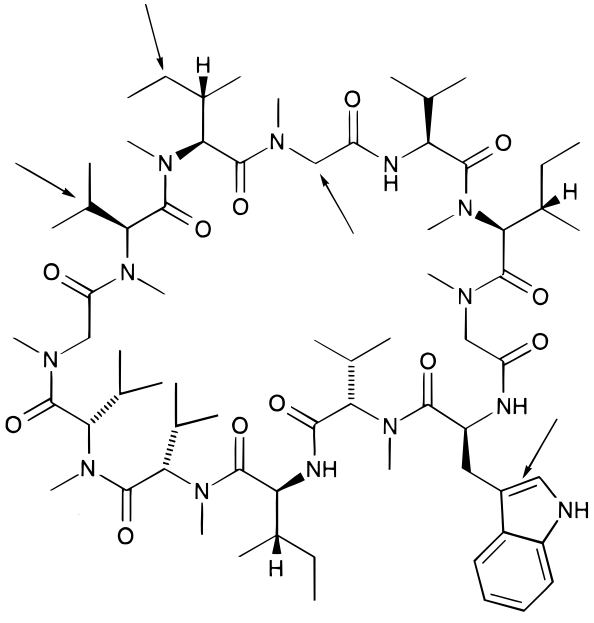

Fig. 1. Structure of omphalotin (omphalotin A). Arrows indicate the positions in which omphalotins B, C and D have been oxidised or oxidised/derivatised.

* To whom correspondence should be addressed.
E-Mail: Olov.Sterner@orgk2.1th.se
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3 Results and discussion

Due to the fact that nine of the 12 α-nitrogens are methylated, the omphalotins are relatively lipophilic cyclopeptides, and soluble in most organic solvents. However, they can exist in several conformations, and the stability of various conformers is affected by the solvent. In general, the data obtained in deuteromethanol were most suitable for the determination of the structures. In establishing the structure of the 36-membered ring, the long-range ^{1}H-^{1}H (COSY) couplings between the *N*-methyl groups and the α-hydrogens, as well as the long-range ^{1}H-^{13}C (HMBC) couplings between the *N*-methyl groups and the carbonyl carbons and the α-carbons, and between the α-hydrogens and the neighbouring carbonyl carbon and the carbonyl carbon attached to the nitrogen, were crucial. The signals for the 12 carbonyl carbons are very close in frequency in deuterochloroform (as well as in other solvents),[3] and insufficient resolution is obtained in a standard HMBC experiment. However, by decreasing the ^{13}C sweep width to approximately 20 ppm it was possible to resolve all correlations from both the *N*-methyl groups and the α-hydrogen atoms. In summary, all the correlations indicated in Fig. 2 could be observed, and established the macrocyclic ring unambiguously.

The nine groups attached to the α-carbons could also be identified by a combination of COSY and HMBC experiments. Hydrolysis of omphalotin and analysis of the amino acids showed that they are all L-amino

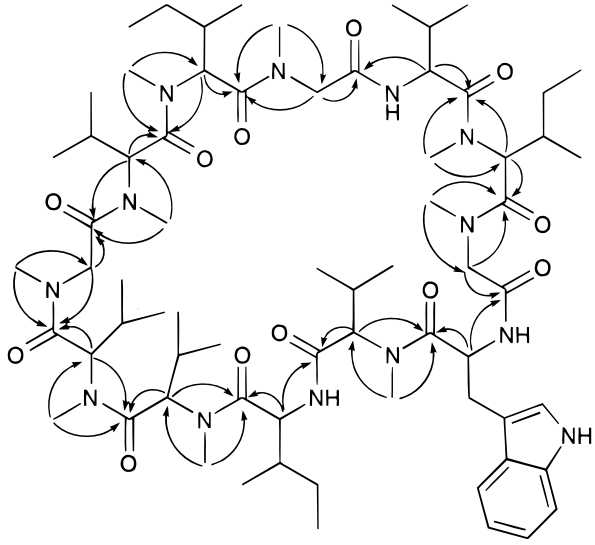

Fig. 2. HMBC correlations observed that establish the molecular frame of the omphalotins.

acids,[2] and the stereostructure presented in Fig. 1 represents the absolute configuration of omphalotin. Although it has not yet been proved, it is reasonable to assume that omphalotin is the biogenetic precursor of the oxidised omphalotins (B, C and D).[2] In fact, the omphalotins B, C and D always appear after omphalotin during fermentations.

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Evidence of an Attractant from Virgin Females of *Bephratelloides pomorum* (Hymenoptera: Eurytomidae): Possible Role of Cuticular Compounds

Ruth R. do Nascimento,* Ivanildo S. de Lima, Cláudia M. L. de Mendonça, Luciano C. N. da Silva, Adriana G. Duarte & Antônio E. G. Sant'Ana

Laboratório de Ecologia Química, Universidade Federal de Alagoas, BR 101 N, Km 14, Tabuleiro, 57072-970, Maceió, Alagoas, Brazil

Abstract: The Annona seed borer, *Bephratelloides pomorum* (Hymenoptera: Eurytomidae), is the most important insect pest of soursop, *Annona muricata* L. The female lays eggs directly into the most recently formed seeds of developing fruit where the larvae remain feeding until pupation. When fully developed, the young wasps make a channel to emerge from the fruit, ready to copulate. Males are attracted to females and display a peculiar behaviour which consists of three steps: antennation, lateral bouncing and wing vibrations. Experiments carried out in semi-field and laboratory conditions showed that males also behave similarly in the presence of filter paper impregnated with a hexane extract of the female's body, suggesting that female *B. pomorum* produce an attractant which enables males to find them. The hexane extracts of females, analysed by GC-MS, were shown to consist mainly of a mixture of straight- and branched-chain hydrocarbons and esters.

Key words: *Annona* sp.; *Bephratelloides* sp.; GC-MS; attractants

1 Introduction

In the north-east of Brazil, the Annona seed borer *Bephratelloides pomorum* (Hymenoptera: Eurytomidae) is the most important insect pest of soursop, *Annona muricata* L. The female oviposits into the most recently formed seeds of developing fruit, which serve as food for the larvae. When fully developed, the young wasps start to excavate an escape tunnel towards the surface of the fruit; this may also result in indirect damage, since fungi and bacteria may invade the fruit through the escape holes made by adult wasps. As a result some attacked fruits fail to develop, they darken, mummify and drop.[1,2] Several authors have reported that wasps of the genus *Bephratelloides* are able to reduce fruit production by more than 70%.[3]

In some species of parasitic wasp, such as *Apanteles* (=*Cotesia*) *liparidis*, and *Cardiochiles nigriceps* Vier., females are responsible for the production of sex pheromone, which helps males to find them.[4,5] However, very little is known about the biology and mating behaviour of *B. pomorum*. Therefore, we decided to study the mating behaviour of wild *B. pomorum* in order to understand the mechanisms involved in the reproductive behaviour of this species.

2 Materials and methods

2.1 Insects. Wild adult wasps of *B. pomorum* were obtained from infested soursop fruits from a commercial orchard in Maceió, Alagoas (north-east Brazil). The fruits were taken to the laboratory and kept in wooden cages until adult emergence. The adults were segregated by sex and kept in separate cages until used.

2.2 Field-cage experiments. The mating behaviour of *B. pomorum* was observed in a field-cage (2 m^3) made from 20-mm nylon mesh. One potted host tree (height 1·6 m) was placed inside the field cage and two infested soursop fruits were hung from branches using cotton string. The fruits were replaced every three days. The average conditions of temperature and relative humidity inside the field-cage were 26($\pm$2)°C and 80($\pm$10)%, respectively. The experiment was replicated 10 times. For each replicate, 20 newly emerged male *B. pomorum* were taken from the wooden holding cages, marked on the thorax with one or two small spots of a non-toxic coloured paint (without anaesthesia) and then released into the field-cage. Their behaviour was then monitored over 90 min.

To observe the attraction of virgin male *B. pomorum* to virgin females, two plastic cages, made from recycled Coca-Cola® bottles, were used. One cage was painted with a black dye and its surface was perforated with small holes to resemble an attacked soursop fruit. The second cage was colourless and transparent and non-

* To whom correspondence should be addressed.